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Prevalence and mechanisms of environmental hyperoxia-induced thermal tolerance in fishes

T. J. McArley, D. Morgenroth, L. A. Zena, A. T. Ekström and E. Sandblom

Department of Biological and Environmental Sciences, University of Gothenburg, PO Box 463, 405 30 Gothenburg, Sweden

TJM, 0000-0002-5248-2828; DM, 0000-0002-3167-4425; ATE, 0000-0002-9966-8160

Recent evidence has suggested environmental hyperoxia (O_2 supersaturation) can boost cardiorespiratory performance in aquatic ectotherms, thereby increasing resilience to extreme heat waves associated with climate change. Here, using rainbow trout (*Oncorhynchus mykiss*) as a model species, we analysed whether improved cardiorespiratory performance can explain the increased thermal tolerance of fish in hyperoxia (200% air saturation). Moreover, we collated available literature data to assess the prevalence and magnitude of hyperoxia-induced thermal tolerance across fish species. During acute warming, O_2 consumption rate was substantially elevated under hyperoxia relative to normoxia beyond 23°C. This was partly driven by higher cardiac output resulting from improved cardiac contractility. Notably, hyperoxia mitigated the rise in plasma lactate at temperatures approaching upper limits and elevated the critical thermal maximum (+0.87°C). Together, these findings show, at least in rainbow trout, that hyperoxia-induced thermal tolerance results from expanded tissue O_2 supply capacity driven by enhanced cardiac performance. We show 50% of the fishes so far examined have increased critical thermal limits in hyperoxia (range: 0.4–1.8°C). This finding indicates environmental hyperoxia could improve the ability of a large number of fishes to cope with extreme acute warming, thereby increasing resilience to extreme heat wave events resulting from climate change.

1. Introduction

Fish living in shallow aquatic habitats, particularly those that are fully or partially enclosed (e.g. shallow lakes, ponds, streams, estuaries and intertidal rock pools), are regularly exposed to acute warming events. The most extreme of such events occur during heat wave weather conditions and are typically characterized by a daily cycle where water temperature ramps up to a peak over several hours during the day and then cools overnight or on the incoming tide. Although generally considered thermally tolerant, fishes occupying habitats where such acute thermal ramping occurs are under the threat of increasingly frequent and more extreme heatwave events due to climate change [1–3]. Compounding the problem is that behavioural avoidance of high temperatures (e.g. moving to deeper, cooler water) may not be possible in these habitats. An important feature of these environments, however, is that they often support high densities of photosynthetic aquatic plants, algae and seaweeds. Thus, when extreme high temperatures arise (i.e. on hot and sunny days), O_2 levels are likely to become hyperoxic (supersaturated with O_2) due to high rates of photosynthesis [4–6]. Indeed, in a recent review, we identified a number of examples of co-occurring acute warming and hyperoxia in shallow aquatic habitats that would support fish populations [7]. Moreover, a thorough assessment of water oxygenation and temperature in shallow Black

Sea habitats demonstrated the highest water temperatures frequently co-occur with hyperoxia [8]. Although available research is limited, there is increasing recognition that naturally occurring hyperoxia could provide an 'ecological refuge' improving thermal tolerance of fish and other aquatic ectotherms [7–10].

Beyond its ecological relevance, a physiologically based theoretical hypothesis as to why hyperoxia may affect warming tolerance in fish has been proposed. The oxygen and capacity limited thermal tolerance hypothesis places an inability of the cardiorespiratory system to meet tissue O_2 demand at high temperatures as the central pillar dictating the upper thermal tolerance limits of aquatic ectotherms [11,12]. At critical thermal limits, tissue O_2 demand is proposed to exceed tissue O_2 supply, leading to functional tissue hypoxia and time-limited survival dependent on anaerobic metabolism [12,13]. Tissue O_2 supply is commonly assessed as the rate of O_2 consumption ($\dot{M}O_2$), which, according to the Fick principle, is a product of cardiac output (the rate of blood flow to the tissues) multiplied by the arterial-venous O_2 content difference (tissue O_2 extraction from the blood (A-V O_2 content difference)) [14]. In fish, it is proposed that limitations of maximal cardiac output primarily dictate ceilings of tissue O_2 supply capacity during warming exposure [15,16]. A prediction stemming from this is that environmental conditions that increase maximal cardiac output during warming will also improve tissue O_2 supply capacity and may lead to better thermal tolerance. Hyperoxia is one such environmental condition that facilitates improved tissue O_2 supply (i.e. higher $\dot{M}O_2$) at temperatures approaching critical thermal limits in fish [9,17], and there is evidence this is driven by increased cardiac output [18]. While this may explain why hyperoxia improves acute thermal tolerance in some fish [8], a direct link between increased tissue O_2 supply capacity, higher cardiac output, mitigation of anaerobiosis and improved upper thermal tolerance is yet to be demonstrated.

In the first part of this study, using rainbow trout (*Oncorhynchus mykiss*) as a model species, we aimed to establish the mechanistic basis of a putative improvement of acute upper thermal limits of fish in hyperoxia. We predicted that should thermal tolerance improve with hyperoxia, this would be associated with greater scope to increase $\dot{M}O_2$ during thermal ramping and a consequent mitigation of anaerobiosis. While environmental hyperoxia increases arterial O_2 partial pressure (P_aO_2) in fish [7], haemoglobin is normally fully saturated in normoxia [19], theoretically leaving little scope for environmental hyperoxia to increase arterial O_2 content further. Moreover, hyperoxia typically increases venous O_2 partial pressure (P_vO_2) [7,18], which would tend to increase venous blood O_2 content. Thus, in the context of the Fick principle, we predicted that increased $\dot{M}O_2$ with environmental hyperoxia would primarily result from increased cardiac output rather than a higher A-V O_2 content difference. In turn, we predicted that improved cardiac output would be driven by increased cardiac contractility (stroke volume), and that this would be associated with elevated P_vO_2 and therefore an enhanced cardiac O_2 supply due to a steeper O_2 diffusion gradient between the returning venous blood and heart tissue [20]. To assess these predictions, we fitted rainbow trout with a ventral aortic blood flow probe and a venous cannula to allow simultaneous measurements of $\dot{M}O_2$, cardiac function, venous blood oxygenation and blood parameters (lactate and haematology) during thermal ramping to the critical thermal maximum (CT_{max}) under hyperoxia (200% air saturation) or normoxia.

Hyperoxia does not always increase the thermal tolerance of fish examined under controlled laboratory conditions [9,17]. Thus, it is unclear whether a recently proposed idea that natural environmental hyperoxia can enhance the resilience of aquatic ectotherms to more extreme acute warming [8] is relevant in a broad range of fishes and environments. In the second part of this study, we therefore synthesized the existing literature concerning the impact of hyperoxia on acute upper thermal tolerance limits in fish. In doing so, we aimed to assess: (i) whether improved thermal tolerance in hyperoxia is a general response observed in a broad range of fishes and (ii) the magnitude of environmental hyperoxia-induced increases in thermal tolerance in fishes.

2. Methods

(a) Experimental animals and holding conditions

The rainbow trout (mean body mass of 896.5 ± 47.5 g and 901.8 ± 73.9 g at the time of experimentation in the normoxia and hyperoxia treatments, respectively) used in this study were of mixed sex and obtained from a commercial trout farm (Vänneåns Fiskodling AB, Halland, Sweden). Prior to experimentation, they were held in two 400 l tanks supplied with recirculated freshwater (air saturated, approx. 10°C and 12 : 12 h light cycle) for a period of at least four weeks of laboratory acclimation. They were fed commercial aquaculture feed (7 mm, Protec Trout pellets, Skretting, Norway) twice a week, but food was withheld for a period of 3 days prior to experimentation.

(b) Surgery and instrumentation

To measure cardiac output, heart rate and stroke volume, a 2.5 mm Transonic transit-time blood flow probe (L type; Transonic Systems, Ithaca, NY) was placed around the ventral aorta to allow recordings of blood flow. Anaesthesia and surgical methods for fitting the flow probe were identical to McArley *et al.* [21]. The ducts of Cuvier were then cannulated with a PE50 catheter to allow venous blood sampling as previously described by Sandblom *et al.* [22].

(c) Experimental protocol prior to thermal ramping

Following surgery, individual fish were placed into respirometers held in 120 l aquariums receiving a constant flow of approximately 10°C recirculated freshwater from the main holding tank supply. After the fish was placed in the respirometer, an O_2 level of approximately 200% air saturation was established for the hyperoxic treatment by bubbling water with O_2, while the normoxia treatment (approx. 100% air saturation) was maintained by bubbling air. These O_2 treatment conditions were then maintained for the remainder of the protocol. Fish recovered from surgery for approximately 22 h, at which point they were removed from the respirometers and exhaustively exercised under normoxia or hyperoxia by manual chasing for a period of 5 min. They were then returned to the respirometers and allowed to recover for 21 h prior to the onset of thermal ramping. During the time prior to thermal ramping, five approximately 250 µl venous blood samples (approx. 1.25 ml total and approx. 3.3% of total blood volume) had been drawn from which the physiological parameters measured are not reported in the current study; the data pertaining to these samples are reported in McArley *et al.* [21]. The blood sample drawn (sixth sample) and the $\dot{M}O_2$ and cardiac function measured at the end of the

post-exhaustive exercise recovery period are used as routine values at 10°C in the current study (see electronic supplementary material, figure S1 for a visual outline of the experimental protocol). At this point, following exhaustive exercise, $\dot{M}O_2$, cardiac output and all blood parameters measured had recovered to pre-exhaustive resting levels under both normoxia and hyperoxia [21]. Thus, although previously exposed to exhaustive exercise, fish in both O_2 treatments were in a similarly well-rested and recovered state prior to thermal ramping. This allowed us to reduce the total number of research animals used, while maximizing the data collected, in accordance with 3R principles.

(d) Thermal ramping protocol

Thermal ramping involved step-wise increases in temperature, which were achieved by heating the water supply to the respirometers housing fish with a water heater controlled with a thermostat. The mean water O_2 level inside the respirometer throughout the entire thermal ramping protocol was 96.9 ± 0.2% air saturation and 208.8 ± 2% air saturation in the normoxia and hyperoxia treatment, respectively. These are referred as normoxia (approx. 100% air saturation) and hyperoxia (approx. 200% air saturation) for the remainder of this paper. Initially, temperature was increased from 10°C to 15°C and then 15°C to 20°C at a rate of 5°C h^{-1}. At 15°C and 20°C, $\dot{M}O_2$ and cardiac variables were measured for approximately 20 min once temperature stabilized (e.g. temperature was increased from 10°C to 15°C in 40 min; then measurements were taken at 15°C for 20 min). A venous blood sample (approx. 250 µl) was also drawn at the end of the 20°C measurement period. From 20°C, the rate of heating was reduced to 2°C h^{-1}. $\dot{M}O_2$ and cardiac variables were measured for a period of approximately 20 min at 22°C and then for 20 min with every 1°C increase in temperature beyond 22°C. A venous blood sample was also taken at the end of the 24°C and 26°C temperature steps. Thermal ramping continued until the loss of equilibrium (i.e. an inability to maintain a stable, upright body position) occurred for a period of 10 s, which was defined as the critical thermal maximum (CT_{max}) [23]. A final blood sample was taken at CT_{max} prior to the fish being removed from the respirometer and euthanized with a concussive blow to the head.

(e) Respirometry for $\dot{M}O_2$ measurement and data acquisition for cardiac variables

$\dot{M}O_2$ was measured using intermittent stop-flow respirometry [24]. Respirometer design, respirometry data acquisition equipment and software, and calculation of $\dot{M}O_2$ were identical to McArley et al. [21]. Briefly, in the 'closed' measurement phase, the linear decline in water O_2 level (sampled at 10 Hz with a fibre optic probe) within a sealed 10 l PVC respirometer was used to calculate $\dot{M}O_2$ at each thermal ramping temperature step. The R^2 for the slope of the linear decline in water O_2 level within the respirometer was greater than 0.98 for the majority of measurement cycles and never below 0.95. Three 'closed' phase measurement cycles (2–5 min) interspersed with a 'flushing' period (5–8 min) were run at each temperature. Background O_2 consumption was assessed at 10°C at the start of the protocol and at the temperature of CT_{max}. At 10°C, a positive background slope, which likely related to a small increase in temperature (approx. 0.15°C) within the sealed respirometer during 'closed' measurement cycles, was detected. The source of this heat was almost certainly the mixing pump connected to the respirometer. This positive slope, however, was reduced in a linear fashion as temperature increased during thermal ramping, and it often became slightly negative at the highest temperatures. Thus, to estimate background O_2 consumption, a linear regression between temperature and the background slope measured at the start (10°C) and end (CT_{max} temperature) of the protocol was used to calculate the background slope at each thermal ramping temperature. These slopes were then added (positive slope) or subtracted (negative slope) from the measurement cycle slopes used to calculate $\dot{M}O_2$.

The signal from the Transonic blood flow probe was sampled at 10 Hz using identical equipment and software as McArley et al. [21], and the probe was bench calibrated between temperatures of 10°C to 26°C according to the manufacturer's instructions (see Morgenroth et al. [25] for a detailed description of the calibration set-up). The flow probe signal was recorded continuously throughout thermal ramping, but only data pertaining to periods of $\dot{M}O_2$ measurement, once temperature had stabilized at each thermal ramping step, was used to assess cardiac parameters.

(f) Calculation of cardiorespiratory variables

Cardiac output was determined from blood flow data and normalized to body mass (ml min^{-1} kg^{-1}), and heart rate was determined from the pulsatile blood flow measurements. Cardiac stroke volume (ml heart beat^{-1}) was calculated by dividing cardiac output by heart rate. Routine and maximal values for cardiorespiratory variables are reported in this study. Routine values for $\dot{M}O_2$ ($\dot{M}O_{2-ROU}$) are the mean of three measurements taken at each thermal ramping temperature step. For cardiac variables, routine values are determined from the mean of three sections of flow trace recorded at the same time as $\dot{M}O_{2-ROU}$ (i.e. routine cardiac variables are tied to $\dot{M}O_{2-ROU}$). Maximal $\dot{M}O_{2-ROU}$ during thermal ramping was taken as the highest $\dot{M}O_{2-ROU}$ (mean of three $\dot{M}O_2$ values) recorded at any temperature. In all fish, this occurred at temperatures of 24°C or higher. Like routine cardiac variables, maximal cardiac variables were tied to $\dot{M}O_2$, such that the maximum values for cardiac output, heart rate and stroke volume reported pertain to the same time when maximal $\dot{M}O_{2-ROU}$ was recorded. Cardiac variables were tied to $\dot{M}O_2$ because a main focus of the experiment was to determine whether predicted differences in $\dot{M}O_2$ between normoxia and hyperoxia were driven by differences in cardiac function. Using the tied $\dot{M}O_2$ and cardiac output measurements, routine and maximal A-V O_2 content difference was estimated by rearrangement of the Fick equation:

$$\text{A-V } O_2 \text{ content difference} = \frac{\dot{M}O_2}{\text{cardiac output}}. \tag{2.1}$$

(g) Blood analysis

Venous blood samples (approx. 250 µl) were drawn prior to thermal ramping at 10°C and during thermal ramping at 20°C, 24°C and 26°C. In each sample, P_vO_2, haemoglobin concentration ([Hb]), haematocrit (Hct) and plasma lactate were assessed using identical equipment and protocols to McArley et al. [21].

(h) Statistics

All analyses were performed using GraphPad Prism (version 9.10), with statistical significance accepted at $p < 0.05$. For repeated measures analyses, a violation of sphericity was assumed and Geisser–Greenhouse adjusted p-values and F-tests are reported. Cardiorespiratory variables ($\dot{M}O_{2-ROU}$, cardiac output, heart rate, stroke volume and A-V O_2 content difference) were analysed in two ways. First, routine responses were compared between normoxia and hyperoxia up to a temperature of 25°C (i.e. prior to any fish reaching CT_{max}) using mixed two-way analysis of variance (ANOVA). For cardiac variables and A-V O_2 content difference, the findings of these analyses are
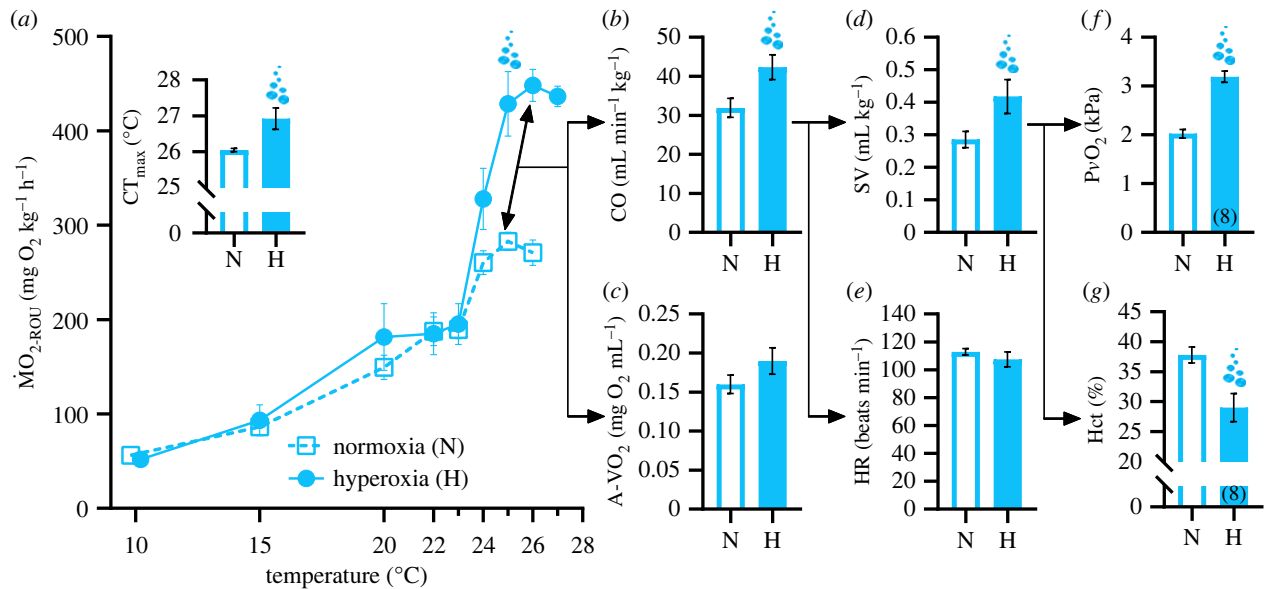
Figure 1. Thermal tolerance and cardiorespiratory performance of rainbow trout (*Oncorhynchus mykiss*) facing acute warming under hyperoxia (200% air saturation) or normoxia. All values are means ± s.e.m. (*n* = 9 unless indicated by bracketed numbers). (*a*) Routine mass-specific O_2 consumption rate ($\dot{M}O_{2\text{-ROU}}$), with critical thermal maximum (CT_{max}: the temperature at which fish could no longer maintain a stable, upright body orientation) shown in the insert. The bubbles in (*a*) indicate a significant difference ($p < 0.05$) in $\dot{M}O_{2\text{-ROU}}$ at 25°C as assessed by mixed two-way ANOVA (see electronic supplementary material, figure S1 for statistical results); (*b–e*) show variables at maximal $\dot{M}O_{2\text{-ROU}}$, which occurred at 24.9 ± 0.26 and 25.7 ± 0.24°C in normoxia and hyperoxia, respectively. (*b*) Cardiac output (CO); (*c*) arterial-venous O_2 content difference (A-V O_2) estimated by the Fick equation; (*d*) cardiac stroke volume (SV) and (*e*) heart rate (HR); (*f,g*) show venous O_2 partial pressure (PvO_2) and haematocrit (Hct) in blood samples drawn via a cannula at 26°C. For the normoxia treatment, seven out of nine blood samples for the 26°C comparison were drawn immediately upon reaching CT_{max}. In the hyperoxia treatment, the eight fish included in the 26°C comparison did not reach CT_{max} for the entire temperature step. The sample size of 8 at 26°C reflects the fact that one fish in hyperoxia reached CT_{max} at 25°C. Bubbles on plots (*b–g*) indicate a significant difference ($p < 0.05$) as assessed by independent-samples *t*-tests, with the exception of CT_{max} and heart rate for which comparisons were made using a Mann–Whitney U test (see electronic supplementary material, table S1 for statistical results). (Online version in colour.)

presented in electronic supplementary material, figure S2. $\dot{M}O_{2\text{-ROU}}$, cardiac output, stroke volume and A-V O_2 content difference were natural log transformed to ensure homoscedasticity and normality of residuals, with the exception of $\dot{M}O_{2\text{-ROU}}$ (figure 1*a*) where the analysis was performed on transformed values despite left skew remaining. In the second analysis of cardiorespiratory variables, maximal $\dot{M}O_{2\text{-ROU}}$ (natural log transformed), cardiac output, stroke volume (natural log transformed) and A-V O_2 content difference (natural log transformed) at maximal $\dot{M}O_{2\text{-ROU}}$, were compared between O_2 treatments using independent-samples *t*-tests (figure 1*b–e*). For heart rate at maximal $\dot{M}O_{2\text{-ROU}}$, medians were compared using a Mann–Whitney *U*-test. Blood variables (PvO_2, Hct, [Hb] and plasma lactate) were also assessed using two analyses. First, at temperatures that all fish reached prior to CT_{max} (10°C, 20°C and 24°C), comparisons were made between O_2 treatments using mixed two-way ANOVA. For [Hb] and Hct, the analysis was run despite non-normality among residuals, as this could not be eliminated through data transformation. The two-way ANOVAs for P_vO_2 and plasma lactate were performed following a natural log transformation to correct for homoscedasticity or non-normality among residuals. The findings of these analyses, with the exception of plasma lactate (figure 2), are presented in the electronic supplementary material, figure S3. In the second analysis of blood variables, independent-samples *t*-tests were used to compare blood samples drawn at 26°C. In the normoxic treatment, this included values from two fish at 26°C prior to CT_{max} and values from seven fish that reached CT_{max} at 26°C (i.e. blood was sampled immediately following the loss of equilibrium in seven fish at 26°C). For the hyperoxic treatment, values at 26°C represented eight fish that did not reach CT_{max} for the entire duration of the 26°C temperature step (note, one fish in hyperoxia reached CT_{max} at 25°C and therefore was not included in the 26°C comparison). Finally, due to non-normality,
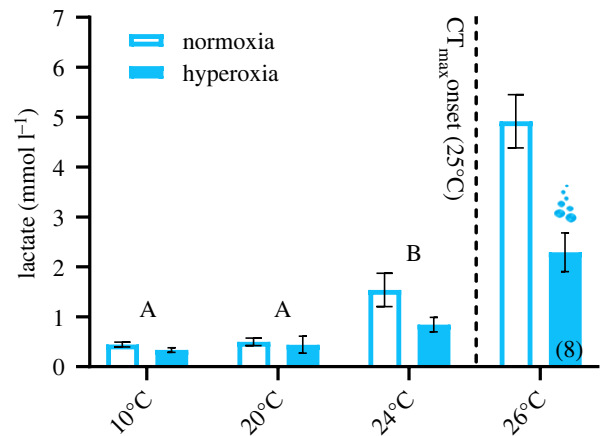

Figure 2. Plasma lactate concentration in rainbow trout (*Oncorhynchus mykiss*) facing acute warming under hyperoxia (200% air saturation) or normoxia. All values are means ± s.e.m. (*n* = 9 unless indicated by bracketed numbers). Over the 10–24°C range, letters represent significant differences ($p < 0.05$) between temperatures across O_2 treatments as assessed by mixed two-way ANOVA (temperature: $F_{(1.53, 24.53)} = 30.95$, $p < 0.001$). At 26°C, bubbles represent a significant difference ($p < 0.05$) between O_2 levels as assessed by an independent-sample *t*-test (see electronic supplementary material, table S1 for statistical results). For the normoxia treatment, seven out of nine blood samples for the 26°C comparison were drawn immediately upon reaching the critical thermal maximum (CT_{max}: the temperature at which fish could no longer maintain a stable, upright body orientation). In the hyperoxia treatment, the eight fish included in the 26°C comparison did not reach CT_{max} for the entire temperature step. The sample size of 8 at 26°C reflects the fact that one fish in hyperoxia reached CT_{max} at 25°C. (Online version in colour.)

median CT_{max} was compared between O_2 treatments using a Mann–Whitney U-test.

3. Results and discussion

(a) Hyperoxia increases thermal tolerance through boosting maximal tissue O_2 supply capacity

The upper acute thermal limit (CT_{max}) was measured to determine if rainbow trout gain a thermal tolerance advantage of hyperoxic (200% air saturation) water oxygenation. In the case of improved CT_{max} with hyperoxia, we predicted a corresponding increase in tissue O_2 supply capacity. To assess this prediction, $\dot{M}O_{2\text{-ROU}}$ (an estimate of whole-animal tissue O_2 supply capacity) was measured in normoxia and hyperoxia during acute thermal ramping (approx. $2°C\ h^{-1}$) to the temperature at which fish could no longer maintain equilibrium (i.e. a stable, upright body orientation; CT_{max}). In hyperoxia, the CT_{max} of rainbow trout was significantly higher than in normoxia (figure 1a). As predicted, the elevation of CT_{max} with hyperoxia was associated with a greater tissue O_2 supply capacity. Indeed, there was a striking difference in the ability of normoxia and hyperoxia-exposed rainbow trout to increase $\dot{M}O_{2\text{-ROU}}$ beyond 23°C (figure 1a). This was reflected by hyperoxia-treated fish having a 52% higher $\dot{M}O_{2\text{-ROU}}$ at 25°C (figure 1a). Moreover, the maximal $\dot{M}O_{2\text{-ROU}}$ during thermal ramping, which was observed at 24.9 ± 0.26 and $25.7 \pm 0.24°C$ in normoxia and hyperoxia, respectively, was 58% higher in hyperoxia (292.8 ± 6.6 mg $O_2\ kg^{-1}\ h^{-1}$ in normoxia versus 462.1 ± 21.6 mg $O_2\ kg^{-1}\ h^{-1}$ in hyperoxia; $p < 0.001$, see electronic supplementary material, table S1 for statistical results). It is proposed that acute warming limits in aquatic ectotherms are set by temperature-dependent performance ceilings of maximum tissue O_2 supply capacity, and that when this ceiling is reached, survival becomes time limited and increasingly reliant on unsustainable anaerobic ATP production [11,12]. Here, we show that the substantial benefit of hyperoxia to tissue O_2 supply capacity mitigated anaerobiosis at high temperatures. Indeed, at 26°C, a temperature where seven out of nine fish in normoxia but only one of nine fish in hyperoxia had reached CT_{max}, plasma lactate levels—a by-product of anaerobic metabolism—were significantly lower in hyperoxia than normoxia (figure 2). This finding indicates that higher CT_{max} in hyperoxia may have resulted from improved O_2 supply capacity shifting the point at which anaerobic ATP production became unsustainable to a higher temperature. As CT_{max} is marked by a loss of coordination, which likely involves some form of neural impairment, severe anaerobiosis in brain tissue may be a candidate for the proximate cause of loss of equilibrium at high temperatures. In support of this, unsustainable anaerobic respiration (i.e. exhaustion of ATP, depletion of glycogen and marked increases in lactate) in brain tissue was identified as a key characteristic at the loss of equilibrium in hypoxia exposed sculpin species [26].

(b) Hyperoxia boosts heart blood pumping capacity

Limits on the maximum blood pumping capacity of the heart have been proposed as the primary determinant of ceilings in tissue O_2 supply during warming exposure in fish [16]. Moreover, as haemoglobin is normally fully saturated in normoxia [19], hyperoxia is unlikely to increase arterial O_2 content and therefore should not influence A-V O_2 content difference [27]. Thus, we predicted that any expansion of tissue O_2 supply capacity (i.e. higher $\dot{M}O_2$) in hyperoxia during thermal ramping would be driven by higher cardiac output. Confirming this prediction, higher $\dot{M}O_{2\text{-ROU}}$ at 25°C in hyperoxia occurred alongside a 29% elevation of cardiac output at the same temperature ($p < 0.05$; electronic supplementary material, figure S2). This also occurred at maximal $\dot{M}O_{2\text{-ROU}}$, where cardiac output was 33% higher with hyperoxia ($p < 0.05$; figure 1b). Because heart rate was similar between treatment groups across temperatures (electronic supplementary material, figure S1) and at maximal $\dot{M}O_{2\text{-ROU}}$ (figure 1e), the higher cardiac output with hyperoxia likely reflected an increased cardiac contractility. Indeed, a strong trend for higher stroke volume with hyperoxia existed at temperatures beyond 24°C (electronic supplementary material, figure S2), and stroke volume was 46% higher with hyperoxia at maximal $\dot{M}O_{2\text{-ROU}}$ ($p < 0.05$; figure 1d). Increased cardiac contractility with hyperoxia may have been the result of an improved cardiac O_2 supply. The partial pressure of O_2 in venous blood was elevated in hyperoxia across thermal ramping temperatures (electronic supplementary material, figure S3) and was approximately 1.2 kPa higher at 26°C (figure 1f). Although the difference in P_vO_2 between normoxia and hyperoxia was relatively small, it existed in a range (normoxia approx. 2 kPa and hyperoxia approx. 3.2 kPa; figure 1f) below the threshold (approx. 6 kPa) where progressive declines in maximal cardiac output with falling perfusate O_2 partial pressure begin in rainbow trout perfused heart preparations [28]. Thus, as P_vO_2 was below the threshold known to impair maximal cardiac output in situ, it is plausible that the higher in vivo P_vO_2 in hyperoxia could have contributed to improved cardiac performance by steepening the O_2 diffusion between blood entering the heart lumen and the spongy myocardium. Elevated P_vO_2 in hyperoxia was also observed alongside improved stroke volume during thermal ramping in European perch (Perca fluvialitis) [18].

The interpretation made here that enhanced cardiac contractility with hyperoxia is driven by higher P_vO_2 is complicated by two factors. First, rainbow trout also have a coronary circulation, which supplies oxygenated arterial blood directly from the gills to the outer compact myocardium [29]. We have recently observed that arterial O_2 partial pressure (P_aO_2) is approximately 16 kPa higher under hyperoxia (200% air saturation) relative to normoxia following exhaustive exercise in rainbow trout (T.J.M. 2022, unpublished data). Moreover, elevated P_aO_2 is a common response to hyperoxia in almost all fish so far examined [7]. Thus, it is likely that hyperoxia also results in increased P_aO_2 during thermal ramping and that improved contractility may be due to a steepened O_2 diffusion gradient between the coronary blood and the compact myocardium of the heart. The second complicating factor is that hyperoxia also reduced Hct in the current study (from 32% to 25% at 24°C, and from 37% to 29% at 26°C; electronic supplementary material, figure S2; figure 1g). In European seabass (Dicentrarchus labrax), experimental anaemia that reduced Hct from 42% to 20% increased peak cardiac output during thermal ramping by 42% [30]. Thus, some of the approximately 33% increase in peak cardiac output observed here with hyperoxia may have been the result of the lower Hct rather than being solely related to improved cardiac O_2 supply directly influencing contractility. In hyperoxia, due to the

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mitigating influence of increased blood P_{O_2}, it may be that an active reduction in Hct can take place without compromising aerobic performance. The potential benefit of this is that lower Hct reduces blood viscosity and could therefore lower the energetic costs of the heart [31].

A somewhat perplexing finding of our study is that, despite lower [Hb] and higher P_vO_2 (electronic supplementary material, figure S3), there was a trend for a 19% higher A-V O_2 content difference (Fick estimated) with hyperoxia at maximal $\dot{M}O_{2\text{-ROU}}$ (figure 1c). If haemoglobin were fully saturated in arterial blood under both O_2 levels, this finding would be inexplicable. Our working hypothesis is that this is not necessarily the case. In rainbow trout exposed to thermal ramping under normoxia, it is known that haemoglobin O_2 saturation can fall to approximately 75% at high temperatures [32]. Moreover, although haemoglobin O_2 saturation was unaffected, P_aO_2 fell from approximately 18.6 kPa to approximately 9.5 kPa in heat shocked (13°C to 25°C in 4 h) rainbow trout [33]. As noted earlier, we now know that hyperoxia drastically increases P_aO_2 relative to normoxia following exhaustive exercise in rainbow trout (T.J.M. 2022, unpublished data). If this also occurs during thermal ramping, it may afford protection against collapsing haemoglobin O_2 saturation and arterial O_2 content as CT_{max} is approached. This hypothesis remains speculative, however, and follow-up studies measuring arterial oxygenation under normoxia and hyperoxia during thermal ramping are required.

(c) Prevalence and magnitude of hyperoxia-induced thermal tolerance in fishes

Recent evidence, as was the case in the current study, has shown hyperoxia can increase the critical upper thermal limits of fish inhabiting shallow, tropical coastal environments, suggesting photosynthetically driven O_2 supersaturation could increase the resilience of fishes living in such habitats to more extreme heat waves associated with climate change [8]. In our own past work, however, hyperoxia has failed to influence upper critical thermal limits [9,17], indicating hyperoxia-induced thermal tolerance may be species and context specific. To understand the generality of the phenomenon of hyperoxia-induced thermal tolerance, we collated existing literature data from studies that examined the influence of hyperoxia on upper critical limits in fish. Ten publications (present study included) were identified (table 1). These studies included 20 species ranging from exclusively tropical to Antarctic climatic regions. Of the 20 species examined, a significant elevation of CT_{max} with hyperoxia has been demonstrated in nine species (table 1). In one further species, hyperoxia appeared to increase upper thermal tolerance (*Carrisius auratus* +1°C) but no statistical comparison could be made due to experimental design (table 1). The magnitude of improvement in thermal tolerance with hyperoxia ranged from +0.4°C to +1.8°C (table 1). This finding indicates naturally occurring hyperoxia could benefit thermal tolerance in a large number of fishes and potentially improve resilience to more extreme heat wave events due to climate change. A caveat of this conclusion, however, is that almost all studies have failed to replicate naturally occurring hyperoxic episodes. The reason for this is that most have been performed in a purely mechanistic rather than ecological context, where replicating naturally occurring O_2 levels and heating rates have not been a priority. The best effort so far has been that of Giomi *et al.* [8] who matched experimental O_2 levels and heating rates to extensive monitoring data from relevant ecosystems. These authors' study demonstrates the largest benefits of environmental hyperoxia to critical thermal limits among the available literature.

In addition to improving upper critical thermal limits in fish, other benefits of environmental hyperoxia may exist at elevated but sub-lethal temperatures. In the current study, the large increase in maximal $\dot{M}O_{2\text{-ROU}}$ at elevated temperatures with hyperoxia, probably means that hyperoxia also increases aerobic scope (the difference between resting $\dot{M}O_2$ and maximal $\dot{M}O_2$) at elevated temperatures. The same expansion of $\dot{M}O_2$ with hyperoxia at acutely elevated temperatures has also been observed in European perch and two triplefin fishes [9,17]. Aerobic scope is proposed to represent the metabolic performance window within which fish can perform aerobically demanding activities [38]. The basic principle is that constraint or expansion of aerobic scope by a given environmental factor corresponds to a constraint or expansion of the capacity of an organism to perform aerobically demanding activities such as swimming, feeding, digestion, growth and reproduction [39]. In this context, we propose the apparent expansion of aerobic scope in fish facing acute warming exposure under hyperoxia may represent a sub-lethal metabolic refuge that mitigates severe constraints on aerobic performance that would otherwise occur with acute warming under normoxia. A possible trade-off to the proposed benefit of hyperoxia to aerobic performance, however, could be increased levels of oxidative stress. Indeed, it is known that hyperoxia can increase O_2 free radical production and cause oxidative damage to tissues in fish (see [7] for a detailed review of this topic). Future studies assessing the benefits of hyperoxia to aerobic performance and thermal tolerance should also consider whether co-occurring hyperoxia and acute warming also impose harmful oxidative stress.

(d) Conclusions

Recent evidence has demonstrated that naturally occurring environmental hyperoxia can improve upper critical thermal limits in fish and therefore may increase resilience of fish living in heat-vulnerable habitats (e.g. rock pools, shallow estuaries and shallow lakes and ponds) to more extreme acute warming events occurring with climate change. Here, we show hyperoxia also increases CT_{max} in rainbow trout. We demonstrate hyperoxia substantially increases maximal tissue O_2 supply capacity at elevated temperatures approaching upper critical limits and mitigates anaerobiosis. Moreover, the blood pumping capacity of the heart is boosted with hyperoxia as evidenced by increased cardiac output and stroke volume. Together these findings indicate that hyperoxia can benefit acute thermal tolerance in fish through expanding cardiorespiratory performance and improving tissue O_2 supply capacity. Our literature review found that environmental hyperoxia increases upper critical thermal limits in half of the fishes so far examined. Thus, naturally occurring environmental hyperoxia could improve upper acute thermal tolerance limits and increase resilience to extreme heat wave events resulting from climate change in a large number of fishes.

Table 1. The effect of environmental hyperoxia on upper thermal tolerance limits in fish. CT_{max} difference = CT_{max} in hyperoxia - CT_{max} in normoxia (a positive number shows higher CT_{max} in hyperoxia), na = no statistical comparison available, ns = not stated. Note: Giomi *et al.* [8] reported the temperature at which 50% of fish became unresponsive (LT50) as a measure of thermal tolerance. As LT50 was determined by sigmoidal regression, the separation of 95% confidence intervals between the regressions in normoxia and hyperoxia was taken as a statistically significant difference. All other studies reported CT_{max} (i.e. the temperature of loss of equilibrium). Ecotype: FW = freshwater, M = marine, BW = brackish water. Climatic region was determined from the latitudinal distribution listed for each species on FishBase (https://www.fishbase.de/).

species	ecotype	climatic region	acclimation temperature (°C)	heating rate (°C h⁻¹)	O₂ level (% air saturation)	CTmax difference (°C)	p < 0.05	reference
Carassius auratus	benthopelagic; FW/BW	subtropical-temperate	17	109	200	+0.9	na[a]	[13]
C. auratus			17	109	450	+1	na	
C. auratus			27	73	200	+0.21	na	
C. auratus			27	73	450	+0.81	na	
Fundulus notatus	benthopelagic, FW	subtropical-temperate	30	20	160	−0.01	no	[34]
Notropis lutrensis	benthopelagic, FW	subtropical-temperate	30	20	160	−0.53	no	
Pimephales vigilax	benthopelagic, FW	subtropical-temperate	30	20	160	−0.16	no	
Fundulus heteroclitus	benthopelagic, FW/M/BW	subtropical-temperate	15	18	ns	+0.3	no	[35]
Perca fluviatilis	benthopelagic (Biotest population), FW/BW	temperate	23	2	200	+0.6	no	[17]
P.fluviatilis	benthopelagic; FW/BW	temperate	17	2	200	+1.1	yes	[18]
Chaenocephalus aceratus	benthopelagic; M	Antarctic	0.5	4	240	+0.12	no	[36]
Notothenia coriiceps	benthopelagic; M	Antarctic	0.5	4	240	+0.74	no	
Bellapiscis medius	benthic (intertidal), M	temperate	21	2	200	+0.13	no	[9]
Forsterygion lapillum	benthopelagic (intertidal/subtidal), M	temperate	21	2	200	+0.43	yes	
Atherinomorous sp.	pelagic, M	tropical	20	2	140	+1.4	yes	[8]
Dascyllus sp.	reef associated, M	tropical	20	2	140	+1.8	yes	
Apistogramma borellii	benthopelagic; FW	tropical-subtropical	31	12	200	+0.66	no	[37]
Brycon amazonicus	benthopelagic; FW	tropical	31	12	200	+1.4	yes	
Carnegiella strigata	pelagic; FW	tropical	31	12	200	+0.51	yes	
Colossoma macropomum	benthopelagic; FW	tropical	31	12	200	+0.08	no	
Corydoras pulcher	benthopelagic; FW	tropical	31	12	200	+0.81	no	
Corydoras schwartzi	benthopelagic; FW	tropical	31	12	200	+0.48	yes	
Paracheirodon axelrodi	pelagic; FW	tropical	31	12	200	+0.41	yes	
Oncorhynchus mykiss	benthopelagic; FW/M/BW	temperate	10	2	200	+0.87	yes	current study

[a]Weatherley [13] did not test statistical significance when comparing CT_{max} but did show a significant increase in the time fish survived exposure to 40°C under hyperoxia relative to normoxia.

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